HISTOLOGICAL OBSERVATIONS ON PANCREATIC SECRETION

by

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Histological Observations on Pancreatic Secretion.

In this paper I wish to record some observations which I have recently been making concerning the behaviour of the zymogen granules of the pancreatic cells during secretory activity—observations which appear to bear out some recent speculations upon the nature of cell mitochondria, and also the supposed double secretion of trypsin, not only into the alveolar ducts, but also into the blood stream.

If portions of the pancreas of a freshly-killed guinea-pig are removed and fixed immediately in osmo-chromic fixative, and after sectioning, stained by Heidenhain's iron haematoxylin method, there are to be observed with great clearness within the alveolar cells numerous rounded, deeply staining zymogen grains, as well as numerous long, filamentous bodies, the mitochondria. If the sections are treated with borax carmine, instead of haematoxylin, neither these granules nor the filamentous bodies take the stain, but the former are still to be recognized by their sharp refractivity. If the sections are treated with safranin, both the granules and the filaments are coloured red. If such sections are examined unstained, the granules are coloured a pale brown, and stand out, though not very clearly, on a lighter background of cell cytoplasm. This suggests their partial lipoidal nature, though their capacity for absorbing haematoxylin or safranin indicates that they are not solely lipoidal.

If on the other hand the tissue is fixed with Bouin's fixative (which contains acetic acid), or with absolute alcohol, the granules, though still present, are no longer capable of staining either with haematoxylin or safranin. The above may then be considered to be the staining reactions of these cell granules.

In preparations of this kind I have frequently been able to observe stages in the secretion of the zymogen granules into the small ducts of the acini, and they frequently may be observed here, in thin sections, in large quantities. They do not as a rule fuse with one another, nor do they immediately dissolve in the fluids of the acinar ducts, but on the contrary are to be observed still, as minute rounded

globules, similar in appearance to the intracellular granules. I have never found them in the larger ducts, which suggests that they speedily dissolve.

The identity of such granules with the granules of the acinar cells is suggested by the following observations. They have the same staining reactions as the intracellular granules (as above described); furthermore, when a portion of the tissue in which secretion has occurred is examined, it is found that the cells are relatively deficient in zymogen granules, or may be lacking them altogether, whilst the acinar ducts are packed with them. This fact has already long been known, of course; Arnold speaks of it as the exhaustion stage.

Now in such preparations I find the filament bodies (mitochondria) also are absent. Furthermore, in resting pancreatic cells, or in cells which are actively secreting, the mitochondria are to be seen breaking up into smaller granules, which cannot be distinguished from the actual zymogen granules. These facts seem to be very strong evidence in favour of the view that the mitochondria are either identical with or closely related to the zymogen grains. There can be little doubt that the latter represent, in part at any rate, the tryptic secretion, dissolved possibly in lipoidal material, whence it follows that the filamentous mitochondria are likewise in the nature of an enzyme. The enzymatic nature of the mitochondria has frequently been referred to. Arnold (1) has observed the breaking up of mitochondria into zymogen grains in the pancreas, thus inferring their relationship to the actual enzymes. Schäfer (4) makes the following remarks upon them: "Pancreas cells frequently exhibit a rounded mass of mitochondria near the nucleus, which is known as the paranucleus; it is probably related to the secretory activity of the cells, and it is not peculiar to the pancreas, although often better marked in that organ than elsewhere."

More recently Marston (3), taking into account their reactions with the azine dye-stuffs, especially with Janus green, has introduced an independent line of evidence, indicating that they contain proteolytic enzymes, whose function it is

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DESCRIPTION OF PLATE.

Sections of pancreas of guinea-pig; osmo-chromic fixation; stained with Heidenhain's iron haematoxylin.

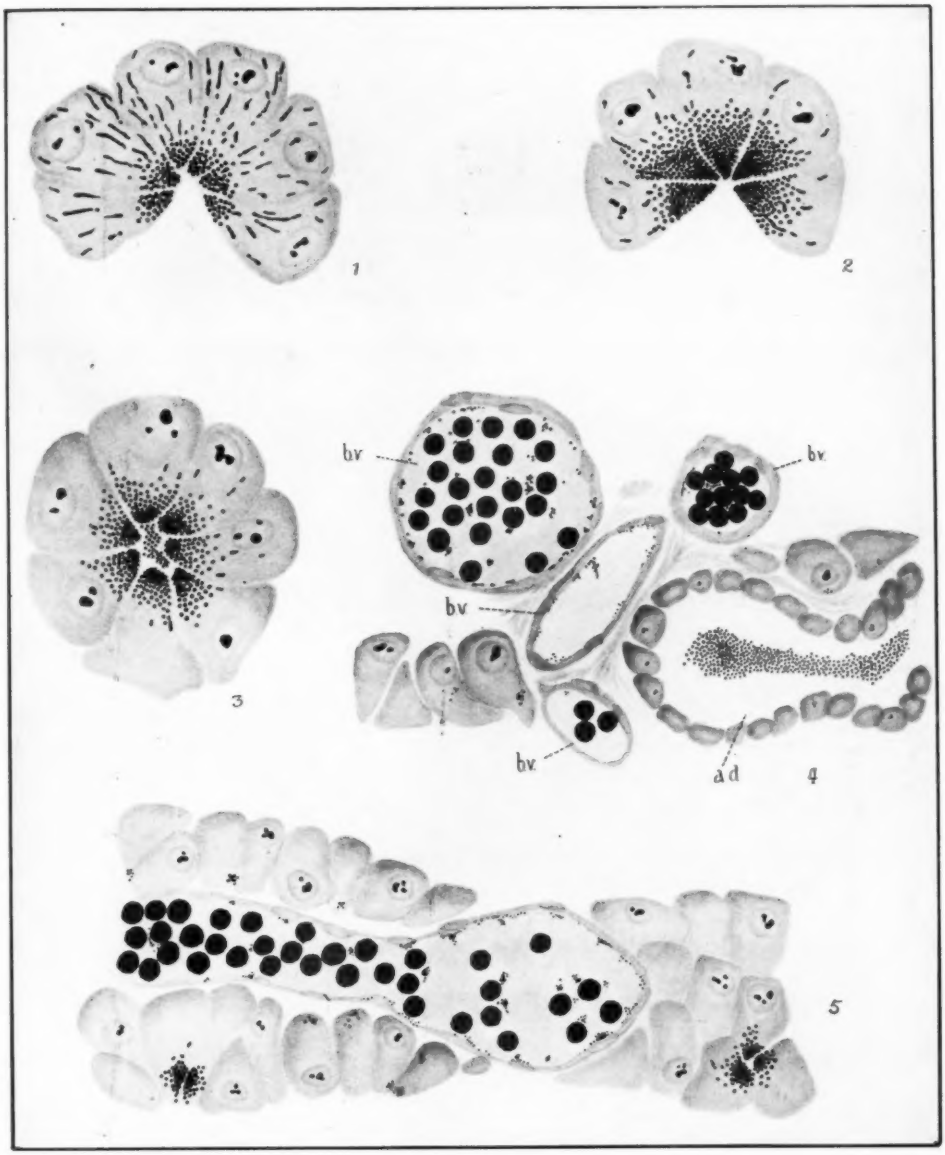
Fig. 1. Alveolar cells, showing filamentous mitochondria giving rise to zymogen grains. (X 1600.)

Fig. 2. The same; later stage, showing almost total disappearance of filamentous mitochondria. (X 1600.)

Fig. 3. The same; filamentous mitochondria have entirely changed into zymogen grains. Note the secretion of whole granules into alveolar duct. (X 1600.)

Fig. 4. Section of pancreas, showing four blood vessels, and one alveolar duct, all containing secreted grains. In one of the blood vessels corpuscles are absent.
b.v., blood vessel; a.d., alveolar duct.
Note the accumulation of granules in the blood vessels, mainly, but not entirely, at the periphery. (X 860.)

Fig. 5. The same, showing a blood-vessel in longitudinal section, containing zymogen granules, mainly, but not entirely, at the periphery of the vessel.
Note the comparative absence of zymogen granules in the pancreatic cells adjacent to the blood vessel. (X 860.)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to build up cell protoplasm by the synthesis of protein in a water-poor phase at their surfaces. On the other hand, Scott (5) believes that they break up into fat globules. I have not been able to make any observations which might serve to confirm this view.

In a number of preparations of guinea-pig pancreas I was surprised to observe these granules, not only within the acinar ducts, but also in the lumina of adjacent blood vessels. If good preparations of pancreatic tissue fixed with the osmo-chromic mixture are made, they are often to be observed within the blood vessels with extraordinary clearness. Several such cases are shown in figures 4 and 5. The blood vessels are often so crowded with these granules that it is difficult to see how they could enter a moving blood stream in such concentration. It might be suggested that a partial blood stasis occurs, which would account for such a great accumulation of granules in the stream. As a further explanation one might offer the fact that even in a rapidly-moving stream of fluid through a tube there is a comparative lack of current at the periphery. The granules are indeed often found in large numbers around the periphery of the vessels, and their uniform occurrence throughout the lumen, which I have often observed (see figures 4 and 5), may possibly be merely a post-mortem effect. In ordinary blood smears, fixed in osmo-chromic, and stained with iron haematoxylin, they cannot be seen.

Dr. Gordon Cameron, to whom I showed my preparations, immediately drew my attention to the recent work of Epstein. This investigator brings forward experimental evidence to prove that trypsin is secreted not only into the alveoli of the gland, but is also liberated into the pancreatic blood vessels. He finds that trypsin is able to combine with insulin and neutralize it by forming an insulin-trypsin compound. He believes that internally secreted trypsin is able to regulate the activity of the islet secretion simply by combining with it. Now the histological appearance of the pancreas certainly lends strong support to the idea of an internal as well as external secretion of trypsin. Concerning the nature of the granules that occur within these blood vessels, I find that they have the same staining reactions as the zymogen grains. When fixed in osmo-chromic fixative they stain deeply with iron haematoxylin or with safranin, but not with borax carmine. Pieces of tissue fixed with Bouin's fixative (which contains acetic acid), or with absolute alcohol, show the same granules in the blood vessels, but they are no longer capable of staining with safranin or haematoxylin.

The pancreatic cells adjacent to such a blood vessel, containing granules, are always very deficient in zymogen grains, having evidently recently lost them. I have observed these granules in four out of twelve guinea-pigs' pancreas examined, which would seem to show that secretion is a periodical phenomenon. So far as I have been able to observe, they are found in the blood vessels only in those cases in which they occur also in the acinar ducts.

SUMMARY.

1. The zymogen grains of the pancreatic cells of the guinea-pig are secreted as grains (globules) into the acini, where they are rapidly dissolved. They arise in the cells mainly from the filamentous mitochondria. This suggests the enzymatic nature of the latter. They also seem to contain a lipoidal substance.

2. The zymogen grains may be secreted, not only into the acinar ducts, but also into the pancreatic blood vessels.

I am greatly indebted to Dr. O. W. Tiegs, of Melbourne University, for his help and advice, which enabled me to carry out this work.

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THE SOURCE OF THE PROTEIN IN THE ALBUMINURIA OF EXPERIMENTAL NEPHRITIS

by

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Cushny (1) has summarized the evidence in favour of the view that most of the protein which passes into the urine in various forms of albuminuria does so through the glomerular capsule. In proteinuria caused by the intravenous injection of egg albumin or haemoglobin, in the albuminuria of cardiac failure and nephritis, and in that which results from temporary obstruction of the renal artery, damage to the glomerular capsule appears to play the dominant role. The convincing nature of this evidence has led to the belief that in albuminuria the protein is derived wholly from the plasma. This conclusion is supported by the direct evidence of many immunological experiments, among which some of the most impressive are those of Salus (8), who found that of ten urines from cases of nephritis, nine reacted with rabbit serum containing precipitating antibodies for human blood serum in accordance with the amount of protein which they contained. Protein derived from these urines could be used to make precipitating sera which reacted with human serum protein. The sera of rabbits immunized with human serum gave no precipitation with kidney protein, and that of rabbits injected with kidney protein gave neither precipitation nor complement fixation when tested with blood serum or urinary protein. In anaphylactic experiments guinea-pigs sensitized with urinary protein or with serum did not react with blood-free kidney protein, and those sensitized with kidney protein were only slightly affected by the injection of serum or urinary protein. Nearly all the immunological evidence upon this question is open to the criticism that insufficient attention has been paid to the fact that organ specificity is not absolute. The experiments of Salus have failed to convince Fischer (5), who still thinks that albuminuria represents a solution of the previously insoluble kidney protein by abnormal accumulation of acids, alkalis, amines, or other substances in the parenchyma, and attributes to lack of specificity the immunological reactions which appear to identify urinary with serum protein. In this connection the experiments of Pearce, Karsner, and Eisenbrey (7) are

important. Using as antigens the serum and albumins, globulins and nucleoproteins derived from the perfused liver and kidney of the dog, they were unable to show any organ specificity in precipitin tests against the sera of immunized rabbits. Anaphylactic experiments made by the intra-venous injection of guinea-pigs sensitized with these antigens showed that while animals sensitized with liver proteins reacted more actively to these than to kidney proteins, and those sensitized with kidney proteins were most affected by proteins from the same organ, all these sensitized animals reacted just as actively with serum.

Fleisher and his colleagues (6) have recently shown by means of crossed complement fixation and absorption experiments that extracts of the liver and kidneys of the guinea-pig possess relative specificity. We have used this method, and also the anaphylactic response of the isolated uterus of the guinea-pig to investigate the nature of the protein in the urine in uranium nephritis. We have already recorded (4) our findings in regard to organ specificity in the experimental animal which we used (the cat). Briefly, we found that extracts of perfused liver and renal cortex were indistinguishable in anaphylactic experiments, though relative specificity could be easily shown by cross fixation and absorption. On the other hand, either extract could be distinguished from serum by tests with the plain muscle of sensitive guinea-pigs.

If, as Fischer has suggested, the positive immunological reactions given by protein in the urine of nephritis were non-specific, this might be made evident by a comparison of the amounts of coagulable protein in the doses of serum and urine respectively necessary to cause the same degree of contraction in the two uterine horns of a female guinea-pig sensitized by the injection of serum. The method, as might have been expected, was not sufficiently accurate for this purpose, because, although the uterine horns are, as Dale (3) has shown, of approximately equal sensitiveness, they are only capable of detecting large differences of concentration of the specific antigen. Further, the protein to be estimated consists of both albumin and globulin, which are not necessarily present in the serum and urine in similar proportions. Finally, there are present in urine substances which have a stimulant action on the plain muscle, and though these may be removed by dialysis, there may still remain others which, without otherwise affecting it, may impair the specific response of the sensitive muscle.

Owing to the difficulty of procuring fresh normal human kidney for the purpose of making extracts, we elected to study uranium nephritis in the cat. Nephritis was produced by the subcutaneous injection of from 8 to 15 mgms. of uranium nitrate into cats of from 2 to 3 kilos in weight. The animals were kept in metabolism cages, and the urine was collected, filtered, and dialyzed against running water for twenty-four hours under a pressure of 200 mm. of mercury in celloidin thimbles. In this way it was possible to obtain concentrated solutions of urinary protein with a very low content in salts, and practically free from substances with a directly stimulant action on plain muscle.

Of five animals injected with uranium, three died within a week or ten days. They showed well-marked tubular nephritis, with but little glomerular or interstitial change. The cells lining the tubules were swollen and vacuolated. There was some necrosis of the lining cells in a few areas, and hyaline and granular casts were present in abundance. In one animal, by repeated injections we produced changes of a more chronic description, though for the most part the glomeruli were normal and the interstitial changes slight in extent and patchy in distribution. The cells lining the tubules had conspicuously ragged edges, and there were many areas of dilated tubules with flattened epithelium. A few glomeruli showed peri-capsulitis, and a few were completely destroyed with hyaline changes in the tuft and corresponding areas of atrophic tubules. The urine tested was obtained as a rule from the second to the fourth day after an injection.

The dialyzed urine from a cat which had had several injections of uranium nitrate, and in which the more chronic changes described above were later found to be present, as well as that from an animal which died a week after the injection with acute nephritis, were tested for their power to fix complement with the sera of rabbits repeatedly injected with cat serum, and also with sera from rabbits injected with extracts of perfused liver and renal cortex of the cat. The method of immunizing these animals and the preparation of the organ extracts is fully described in our earlier paper (4). The cat serum, organ extracts, and urinary protein were used in fixed dilutions, in which they were shown to have no anti-complementary action, and the extent of complement fixation was estimated by using a series of dilutions of the rabbit sera.

These experiments were unsatisfactory, since we were unable to produce rabbit sera with a high complement fixing titre for cat serum. The sera which we obtained from rabbits by repeated injections of cat serum only fixed complement with this antigen in low dilutions (1 in 2, 1 in 5), and behaved similarly with concentrated urinary protein. On the other hand, sera with a high titre for cat kidney and cat liver extracts (fixing complement with these antigens in dilutions of the serum of 1 in 200 and 1 in 40 respectively) did not fix complement in any dilutions with either serum or urinary protein. These results, as far as they go, favour the view that the urinary protein from these cats was mainly derived from the plasma.

In our anaphylactic experiments, however, we were able to produce definite evidence of the presence of soluble protein (other than that derived from the plasma) in the urine of some animals with uranium nephritis. Since the anaphylactic plain muscle appears to be incapable of differentiating between liver and kidney protein, we cannot affirm with certainty that this protein is of renal origin, though this is in the highest degree probable. Our results cannot be due to the presence of casts and epithelial debris from the kidney, since these were removed by filtration before dialysis, though they may be explained by digestion of such cellular material.

Virgin female guinea-pigs were sensitized to cat serum by the injection of 0·5 cc. of cat serum, and others by the subcutaneous implantation of pieces of thoroughly perfused kidney cortex. Three or four weeks after the sensitizing injection or implantation the animals were killed, bled out, and their uteri tested by the method of Dale and Laidlaw (2) in a bath of 120 c.cm. capacity. The only modifications of Dale and Laidlaw's technique were perfusion of the uteri with cold instead of warm Ringer and the storage of the second horn in the cold till needed. We usually tested the condition of this second horn by adding 0·01 mgm. of histamine to the bath after relaxation had occurred before proceeding with the experiment. We were also careful to use doses of urinary protein, serum and kidney extract which were not stimulant to normal plain muscle.

We have selected from our numerous experiments some which were made with the second and fourth day urine from a cat weighing 2·5 kilos, which had received 8 mgms. of uranium nitrate subcutaneously. The urine was filtered and dialyzed for 24 hours, and 44 cc. of each of the 24 hours' samples was concentrated and made up to 8·1 cc. The filtered urine of the second day contained 0·081% of protein, estimated by Kjeldahl on the precipitate obtained by heating after the addition of acetic acid, and that of the fourth day 0·089% coagulable protein.

The kidney extract and serum used in the experiments were obtained from a cat weighing 2 kilos, which was bled out from the abdominal aorta with full aseptic precautions under ether anaesthesia, and in which the kidneys were decapsulated and perfused with 10 litres of sterile saline by the method described in our earlier paper. Twenty grams of cortex was obtained, which was pounded in a mortar, and an extract made by the addition of 44 cc. of sterile saline. The extract, after standing in the cold for a few hours, was cleared by centrifugation, and kept at 4°C. till required. It contained by Kjeldahl 1·26% of coagulable protein. The serum from the same animal contained 7·67% coagulable protein. No preservative was added to the extract, and our procedure in testing any sample of urine was to carry out all the experiments continuously in the course of about 48 hours, for which time both the urine and the extract kept well, and did not develop any stimulant action on the smooth muscle.

Experiments 1 and 2 were made with the second day sample of urine, and show clearly the presence of protein other than that derived from the plasma. They also illustrate the variation in the sensitiveness of guinea-pigs sensitized by implantation of kidney cortex. This method of sensitizing guinea-pigs was much more certain than that of single or repeated injection of extracts, probably because a continuous absorption of antigen takes place for some days after the implantation. The implantations were done under ether anaesthesia, with full aseptic precautions, by making an incision in the skin and introducing subcutaneously a piece of sterile perfused tissue about half a cubic centimetre in

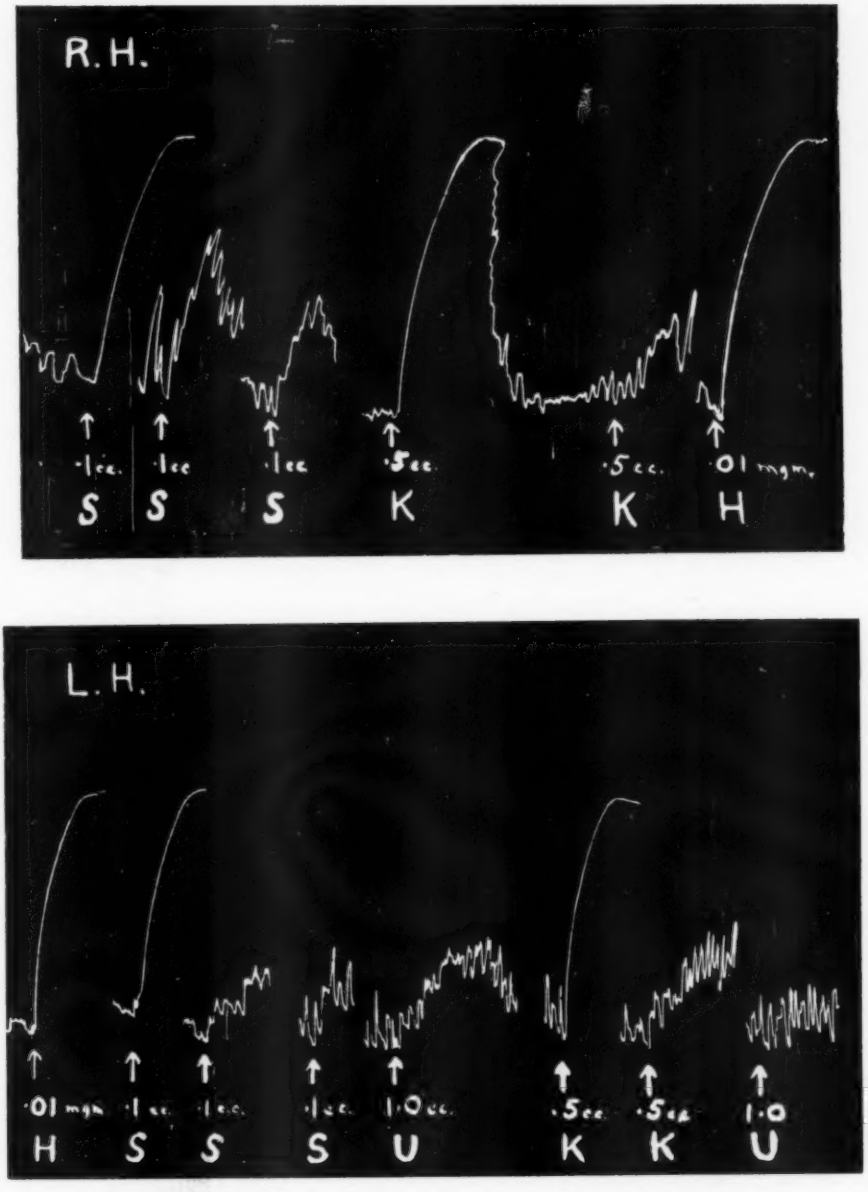
R.H.
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Figure 1.

volume. There was never any suppuration, and the wound invariably healed by first intention. After three weeks we found at the site of implantation only a minute bead of necrotic debris walled off by fibrous tissue.

EXPERIMENT 1. (Figure 1.)

Guinea-pig 130. Weight, 252 gms. Sensitive 23 days to cat kidney cortex.

Right Horn. After a maximal response to 0·1 cc. of serum and smaller ones to two subsequent doses, 0·5 cc. of kidney extract caused a maximal response, and the plain muscle was then partially desensitized, as was shown by the small response to a subsequent dose of extract. The extent of a maximal contraction was shown by the reaction to 0·01 mgm. of histamine.

Left Horn. After a maximal response to 0·01 mgm. of histamine, the muscle reacted in the same way as the right horn to three successive doses of 0·1 cc. of cat serum. 1·0 cc. of concentrated urine then gave a response which was definitely less than half maximal. This did not cause any appreciable desensitization to 0·5 cc. of kidney extract, which subsequently gave a maximal response. A second dose of kidney extract gave only a small response, and finally the plain muscle failed to react to a second dose of 1·0 cc. of concentrated urine. The Ringer in the bath was changed between each response in this and other experiments.

The experiment illustrates very well the method which we used. The plain muscle was first desensitized, as far as any response to serum protein accidentally present in the kidney extract or in the dialyzed urine was concerned, by the repeated addition to the bath of 0·1 cc. of serum containing nearly 8 mgms. of serum protein. After this treatment it would doubtless have reacted specifically to a smaller dose of kidney extract than 0·5 cc. The sample of urine equivalent to 5 cc. of second day urine contained an amount of protein, other than serum protein, less than that present in 0·5 cc. of kidney extract. The response obtained with this concentrated urine was clearly not due to the presence of any stimulant substance, a second dose after thorough desensitization failing to give any response. The reaction to this dosage of urinary protein was, however, insufficient to cause any obvious desensitization to 0·5 cc. of kidney extract. Another guinea-pig of the same series proved to be less sensitive to kidney extract, and gave a clearer quantitative result.

EXPERIMENT 2. (Figure 2.)

Guinea-pig 132. Weight, 213 gms. Sensitive 23 days to cat kidney cortex.

Right Horn. This gave no response to 0·02 cc. of serum, and only a trivial one to a second dose of 0·1 cc. 0·5 cc. of kidney extract now gave a rather more than half maximal response, as judged by the subsequent response to 0·01 mgm. of histamine.

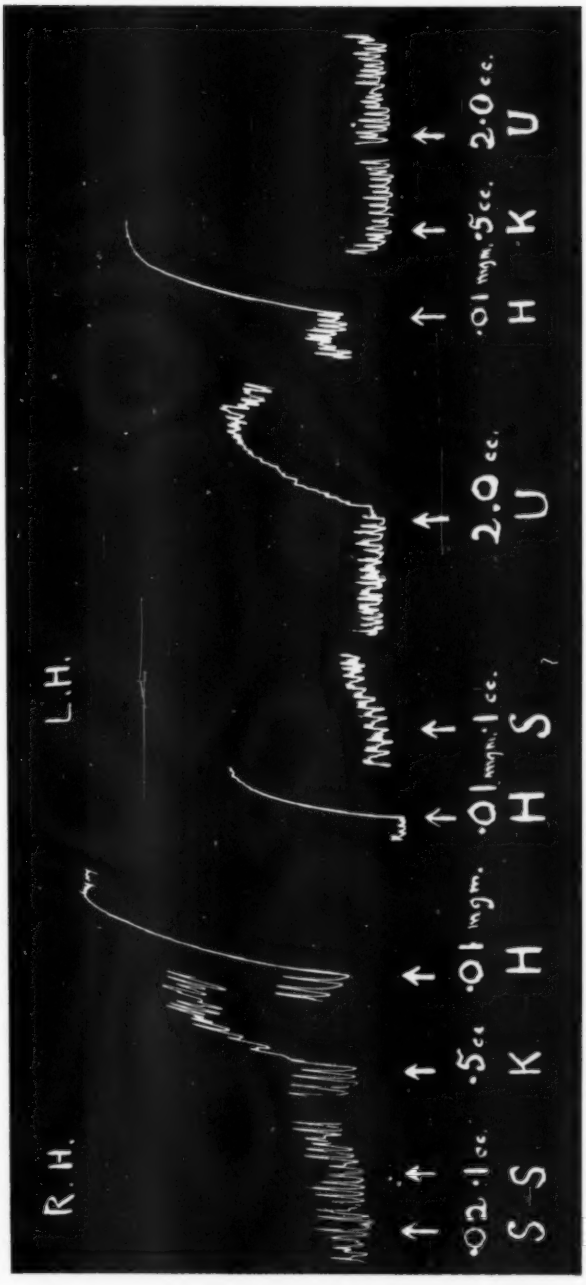

R.H.
L.H.
S S K H H S U U H K U
.02 .1 cc. .5 cc. .01 mgm. .01 mgm. .1 cc. 2.0 cc. .01 mgm. .5 cc. 2.0 cc.

Figure 2.

Left Horn. This reacted maximally to 0·01 mgm. of histamine, and gave only a trivial response to 0·1 cc. of serum. 2·0 cc. of concentrated urine gave a response which was about half maximal, and the plain muscle then failed to respond to 0·5 cc. of kidney extract and to a second dose of 2·0 cc. of urine, though it responded maximally to 0·01 mgm. of histamine.

The response to the urinary protein other than serum protein in 10 cc. of the unconcentrated urine was in this case sufficient to desensitize the uterus completely to a subsequent dose of 0·5 cc. of kidney extract. This dose of urine, containing 8·1 mgms. of coagulable protein, seems to contain an amount of anaphylactically active protein (other than that derived from the serum) of the same order as that present in 0·5 cc. of kidney extract (containing 6·3 mgms. of coagulable protein, a large proportion of which was doubtless inactive nucleoprotein).

Experiment 3 shows the presence of serum protein in this specimen of urine, and though it is not so striking as other experiments, we present it because we excluded any non-specific reaction to protein other than serum protein in the urine by desensitization with kidney extract, which either contained serum protein, despite the careful perfusion, or less probably, shared with it a common antigen.

EXPERIMENT 3. (Figure 3.)

Guinea-pig 135. Weight, 250 gms. Sensitive 22 days to cat serum.

Right Horn. This reacted maximally to 0·01 mgm. of histamine and to 1·0 cc. of kidney extract (containing 12·6 mgms. of coagulable protein). A second dose of kidney extract caused only a small response, and 0·02 cc. of serum gave a less than half maximal response. Finally the response to histamine was shown to be unimpaired.

Left Horn gave somewhat similar responses to repeated doses of 1·0 cc. of kidney extract, and subsequently gave a rather less than half maximal response to 0·75 cc. of urine, which, however, did not desensitize the uterus to a final dose of 0·05 cc. of serum.

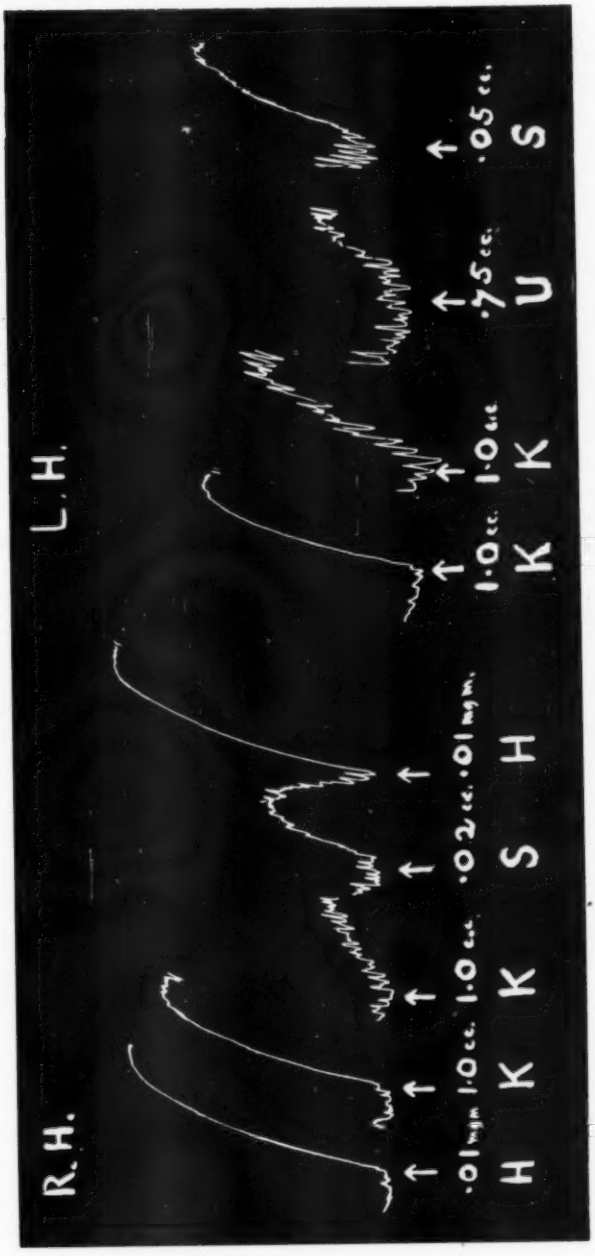
In this experiment we deliberately selected doses of urine and serum which we thought would give sub-maximal responses. The dose of urine used contained 3 mgms. of coagulable protein, and apparently contained an amount of serum protein of the same order as that present in 0·02 cc. of serum (1·5 mgms.).

Experiments 4 and 5 were made with fourth day urine, which was somewhat richer in protein.

EXPERIMENT 4. (Figure 4.)

Guinea-pig 126. Weight, 200 gms. Sensitive 24 days to cat serum.

The *Right Horn* reacted maximally to 0·02 mgm. of histamine and to 0·8 cc.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L.H.
.01 mgm. 1.0 cc. 1.0 cc. .02 cc. .01 mgm. 1.0 cc. 1.0 cc. .75 cc. .05 cc.
H K K S H K K U S

Figure 3.

of urine, which desensitized it to such an extent that it failed to react to a subsequent dose of 0·04 cc. of serum.

The *Left Horn* reacted maximally to 0·02 mgm. of histamine and to 0·04 cc. of serum, which desensitized it sufficiently to prevent it reacting to a second dose of 0·04 cc. of serum.

The urine contained in 0·8 cc., which was equivalent to 4 cc. of the original filtered urine, 3·5 mgms. of coagulable protein, which was about equivalent in

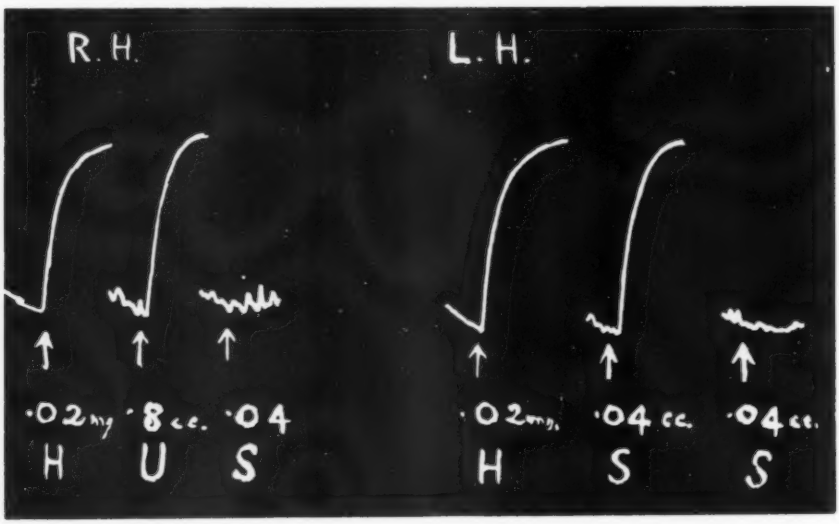

Figure 4.

its anaphylactic effect to 3 mgms. of serum protein. It is probable, however, that the presence of protein in the urine other than that derived from the plasma (as shown in Experiment 5), contributed to its stimulant effect on the sensitive plain muscle.

EXPERIMENT 5.

Guinea-pig 131. Weight, 206 gms. Sensitive 25 days to cat kidney cortex. *Right Horn.* This reacted maximally to 0·02 mgm. of histamine. It gave a sub-maximal response to 0·2 cc. of kidney extract, and was then desensitized, and failed to react to a second dose of extract and to 0·1 cc. of serum, giving finally, when fully relaxed, its maximal response to 0·02 mgm. of histamine.

Left Horn. The left horn reacted maximally to 0·02 mgm. of histamine. It then gave a sub-maximal response to 1·0 cc. of urine, and was desensitized, failing to react to subsequent doses of 1·0 cc. of urine and 0·2 cc. of kidney extract.

The original urine contained in 10 cc. of 8·9 mgms. of coagulable protein, of which there was sufficient antigenically active protein to give a response of the same order as that produced by 0·2 cc. of kidney extract, containing 2·5 mgms. of coagulable protein. In this experiment it is possible that some of the serum protein present in the urine played a part in the response, since the plain muscle was not first desensitized to serum protein.

It is clear from our experiments that in acute uranium nephritis in cats, where the damage is predominantly tubular, there is present in the urine a definite proportion of soluble protein other than that derived from the plasma, and apart from casts and epithelial debris from the tubules. In most of our experimental animals this seemed to be less easily demonstrated than serum proteins, which were invariably present.

Our thanks are due to Miss B. Splatt and Miss Cecil Maudsley, who carried out the protein estimations for us.

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TRIAL AND ERROR AND INTELLIGENCE IN THE BEHAVIOUR OF CERTAIN ARTHROPODS

by

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The work now to be described grew out of some experiments on intelligence in Arthropods, using the word intelligence in the sense in which it is commonly employed by biological psychologists as synonymous with educability, or capacity to learn by experience and so to modify instinctive behaviour in accordance with the animal's past experience in similar situations. These experiments are extensive, and still unfinished. Moreover, a detailed analysis will be required for their interpretation. It is not the object of this communication to present this analysis, but to state some general conclusions, and in particular to describe some experiments on one type of behaviour in water mites.

The animals mostly used in these experiments were the water flea *(Daphnia carinata)*, three or four species of water mites *(Hydrachnidae)*, and the common fresh-water crayfish *(Parachaeraps bicarinatus)*. The principle in all the experiments was the same. The animal was placed in conditions which were certainly unfavourable to its normal activities, such as a confined space, water too shallow to allow of unrestricted swimming movements, or excess of CO_2 dissolved in the water. In every case there were two apparent avenues of escape, one actually leading to freedom, the other ending in a cul-de-sac, so that a choice of this avenue failed to give relief. In many experiments the "unpleasant" result of making the wrong choice of the two possible avenues was reinforced by the administration of an electric shock, or by other means. In the great majority of the experiments the apparatus consisted of a Y-shaped "maze," the animal being placed in the stem of the Y, and having to make a choice between the left-hand or right-hand branch of the Y when it arrived at the point of bifurcation. The size of the apparatus was, of course, adapted to the various sizes of the different animals used.

A dozen experiments with Daphnia, involving altogether over 1,400 trials, or "lessons" (the longest series in any one experiment being 300), failed to disclose any power on the part of this animal to learn by experience. That is to say, the proportion of right to wrong choices showed no tendency to increase

as the experiments proceeded. In the experiment in which one specimen was given 300 trials (extending over eleven days) it made fewer correct choices in the last quarter of the experiment than in any of the other three quarters.

Far more extensive observations were carried out on water mites. Twenty principal experiments were conducted, the number of trials in each experiment varying from 100 to 800, with a total of over 5,000. The first few experiments appeared to give positive results, but further tests undertaken to confirm this showed that while the Hydrachnids have a well-marked tendency to form motor habits (tending to run for a long series of trials into the same passage), there is no good evidence of their power of learning. Thus, while some show improvement during the course of experiment, others show the reverse. In an experiment in which 800 trials were given, and an electric shock was administered on entering the wrong passage, the number of correct choices made in the first 200 trials was 57 (the apparatus being biassed in favour of the wrong passage), in the second 49, in the third 41, and in the fourth 42. I may add that the violent reaction of the animal to the electric shock was sufficient evidence of its "unpleasantness".

The experiments with young crayfish *(Parachaeraps)* gave very different results. This animal, tried in an apparatus of the same type as that used for Daphnia and water mites, but proportionately larger, very soon learnt to avoid the wrong passage and take the right one. In one experiment, in which entry into the wrong passage, besides failing to bring freedom, resulted in an electric shock, the animal took the wrong (left) turn at its first trial, and then chose the right passage seven consecutive times. The electrodes were then transferred to the right-hand passage. The animal continued to go into this passage for 8 more times, receiving a shock each time. In the next 21 times it only twice entered this passage, making the correct choice in the other nineteen. Other experiments in general agreement with this one were carried out with crayfishes, but none of them were on an extensive scale, partly because the positive results of the training were so quickly obvious, and partly because the educability of the higher Crustacea has already been amply demonstrated by Yerkes and others. The experiments with this animal were as a matter of fact only undertaken to verify that the type of apparatus used with Daphnia and the water mites would give evidence of educability in more intelligent animals of the same general type of structure as themselves.

It seems clear then that within the phylum Arthropoda, and indeed within the single class Crustacea, there exists a very wide diversity of intelligence, ranging from an animal which shows no signs of ability to learn a lesson, even after several hundred trials, to one which will learn the same lesson almost perfectly in half a dozen trials.

It seems possible to correlate this difference of intelligence with the mode of life of the animals. The crayfish searches out its food by means of its sense

organs (telereceptors), and having found it, manipulates it with its mandibles and maxillae. It will also defend itself with its chelae, and probably attack other animals that might serve it as food.

Daphnia lives a life of far less initiative. It feeds on microscopic organisms, which are collected by a current of water produced by the movement of certain of its appendages. Presumably there is no need, nor indeed much scope, for searching for food. Unlike the crayfish, Daphnia shows no evidence of awareness of other animals or bodies, except for an acceleration of the swimming movements when disturbed.

The water mites of the genus *Eylais*,[1] on which nearly all the above-mentioned experiments were performed, feed on Daphnia, which they catch as they swim. The method of discovery and capture of their prey involves a minimum of psychological activity, and seems interesting enough to warrant description.

These animals, though possessing eyes and other sense organs which have been interpreted as olfactory, appear to use no other sense than that of touch to discover their prey. If an Eylais is placed in a large vessel of water it displays a ceaseless activity, swimming rapidly round and round, up and down, and turning sharply hither and thither. An animal that has not fed for some time may keep up these movements (very swift for its size) for many hours, without more than a few rests of a second or two duration. A close study of their method of capturing their prey indicates that the object of their almost ceaseless movements is to bring about chance collisions with the Daphnias on which they live. If an Eylais is placed in a small vessel with a few Daphnias, it evidently does not search for them by means of telereceptors. In fact, it appears to be quite unconscious of their presence even at the distance of a millimeter. Whenever, however, in its tireless zig-zag movements a mite collides with a Daphnia, it makes a rapid movement to seize its prey, which may or may not be successful. If the water mite only brushes against the Daphnia as it passes, it swings round in the direction of the Daphnia which it has just touched, and circles round the spot two or three times. In doing this it may collide with the Daphnia again, but much more often it misses it, and resumes its ordinary course. The observer cannot fail to be impressed by the enormous expenditure of energy incurred in this method of discovering their prey by accidental collisions, and also by the frequent failure of the Eylais to secure the Daphnia, even after it has come into collision with it, at any rate, under aquarium conditions.

The following experiments were carried out to test the correctness of these conclusions from the observation of the animal's behaviour.

Specimens of Eylais were placed singly in shallow cylindrical vessels, about 25 mm. in diameter, and holding about 25 cc. of water. In one series one Daphnia was placed in each dish, in a second series two, and in others four, eight, and sixteen respectively. If the mites really discover their prey by chance collisions

[1] I am indebted to Mr. E. J. Semmens for the identification of these forms.

with them, it is plain that the time taken to secure a Daphnia should be inversely proportional, on the average, to the number of Daphnias present.

Twenty tests were made with each of the five numbers of Daphnias, and records kept of the length of time taken to catch one, except where the time exceeded an hour. In twelve out of the twenty tests with only one Daphnia present the water mite failed to catch it within an hour. In only nine out of the twenty tests with two Daphnias present did the mite fail to catch one of them in an hour. With four Daphnias present, three mites failed; with eight Daphnias, two failed; and with sixteen Daphnias, none failed to catch within the hour, the longest time in this series being 32 minutes.

In order to make sure that failure to catch a Daphnia within an hour was not due to the fact that the water mite was not hungry, every specimen that failed was placed at the end of the hour in a small vessel crowded with Daphnias. In every case the water mite caught one within a few seconds. I may add that in most of the experiments the Eylais did not once stop its rapid hunting movements, and in no experiment did any specimen stop for more than a few seconds.

It is not possible to compare directly the average time taken to catch a Daphnia in each of the series, for two reasons: Firstly, we do not know what time to allow for the cases in which no Daphnia was caught in the hour; and, secondly, the number of minutes taken to catch each Daphnia are not distributed at random round a mean, but there is in every series an undue preponderance of quick catches over slow ones. In the series with 16 Daphnias present, for instance, thirteen mites caught a Daphnia within 5 minutes; four caught one in 5-10 minutes, two in 10-15 minutes, and the remaining one in 32 minutes. In other words, the chances of a mite catching a Daphnia in any unit of time diminishes as time elapses. This does not seem to be due to fatigue on the part of the water mites, for as far as observation can show, they are fully as active at the end of the hour as at the beginning. Nor does it appear to be due to differences in degree of hunger, for reasons already stated.

It is, however, possible to institute a comparison in the following way:

With 1 Daphnia present 8 mites caught it within 60 min.; average time to catch, 24·4 min.

With 2 Daphnias present 10 mites caught one within 30 min.; average time to catch, 10·9 min.

With 4 Daphnias present, 10 mites caught one within 15 min.; average time to catch, 5·6 min.

With 8 Daphnias present 10 mites caught one within $7\frac{1}{2}$ min.; average time to catch, 3·3 min.

With 16 Daphnias present 9 mites caught one within $3\frac{3}{4}$ min.; average time to catch, 1·7 min.

Or if we consider the last three series (omitting the first two because of the

small numbers caught within the hour), and average the time taken to catch the first 17 Daphnias (the total caught in the series with four Daphnias present), we get the following result:

	Four Daphnias present. mins.	Eight Daphnias present. mins.	Sixteen Daphnias present. mins.
Mean time to catch ..	17·7	10·8	3·7
Theoretical times ..	18·4	9·2	4·6

The "theoretical times" are on the supposition that the time taken to catch a Daphnia varies inversely as the number present.

Considering both these methods of comparison, therefore, it appears that the statement is justified that the time taken to catch a Daphnia varies inversely as the number of Daphnias present. This supports the conclusion derived from direct observation that Eylais does not make use of telereceptors in searching for its prey, but relies purely upon chance collisions for coming in contact with it. For to an animal in a confined space, tracking down its prey by sight or smell, it would surely make little difference in the case of capture whether there were one, two, four, eight, or sixteen of the prey present. A hungry cat in an enclosed yard would not take eight times as long to catch one out of two chickens as it would to catch one out of sixteen.

SUMMARY.

Intelligence (educability), as estimated by ability to learn to take a right-hand turn leading to relief from unfavourable conditions, and to avoid a left-hand turn leading to further unfavourable conditions, varies greatly within the phylum Arthropoda, and even within the class Crustacea.

This difference in intelligence can be correlated with the degree of psychological development required for the normal life of the animals. This is shown to be extraordinarily small in the case of the active predaceous water mite.

THE NERVE NET OF PLAIN MUSCLE, AND ITS RELATION TO AUTOMATIC RHYTHMIC MOVEMENTS

by

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There exists at present a serious discrepancy in the results of physiological and anatomical investigation concerning the automatic rhythmic movements undergone by isolated strips of plain muscle tissue when deprived of nerve ganglia.

In the old investigations of Engelmann, and the later work of Bayliss and Starling, Gunn and Underhill, and Alvarez, these movements are considered as purely myogenic, since such muscle strips undergo undiminished rhythmic beating even after treatment with nicotine of a strength that is sufficient to paralyze sympathetic synapses; and moreover, while deprival of blood circulation rapidly exhausts nerve ganglia, plain muscle strips, isolated from the body, may undergo spontaneous movements for days (7). The purely muscular origin of these movements has been regarded as firmly established by the observation that rhythmical motion may persist even when all visible traces of ganglia have been removed, or when strips of muscle, normally devoid of ganglia (such as the retractor penis muscle of certain mammals), are employed.

In all these investigations, however, no heed has been paid to the very pronounced nerve net which lies in intimate association with the tissue, and ramifies throughout it, coming into very close relation to the individual muscle fibres. Bethe, in his pioneering work on these nerve nets, ascribed all plain muscle movement, and also the origin of the heart beat, to them. In the case of the medusae, conduction of impulses can be quite definitely proved to be non-muscular, since Bethe has shown that the impulses travel across portions of the animals in which muscle tissue is absent, but in which the nerve net is well marked. But in the case of the rhythmical movements of vertebrate plain muscle no such proof was forthcoming, and the strongest evidence in its favour was the analogy with medusan muscle. In the case of cardiac muscle no facts beyond an analogy with plain muscle could be adduced (1).

In my own work on plain muscle (17) I have had to rely on such a structure as the nerve net to transmit impulses throughout the tissue and co-ordinate its

movements, since no conduction of impulses could be ascribed to the individual plain muscle fibres. This view was greatly strengthened by the fact that in all cases in which such a nerve net was sought, it was actually found, although, it is true, its presence has been denied at times by various authors. I was not aware at the time, however, that certain objections had been raised against the existence of these nerve nets in Bethe's sense; and since a precise knowledge of them is necessary for a clear understanding of plain muscle action, I have devoted some further time to the problem.

A nerve net, in the sense in which Bethe uses the term, is a diffuse, rather open, delicate network, composed of multipolar nerve cells, the processes of adjacent cells being not merely in contact, but directly continuous with one another (figures 1, 2, 5, 6). It is devoid of synapses, and the nerve cells have a structure quite different from that of ganglion cells of the central nervous system, in that Nissl granules, as well as the characteristic cell nucleolus, are absent. Neurofibrils are extremely prominent, and such a nerve net is eminently suited for the diffuse transmission of impulses through the tissue within which it spreads. The intimate relation between the nerve net and the individual plain muscle fibres has never been observed. A nerve net does not occur in skeletal muscle, where the excitation travels along the two excitable membranes of each muscle fibre.

The most adverse criticism of these nerve nets has originated from the work of F. B. Hofmann and his school. Hofmann, using cephalopod material, observed that the nuclei of the nerve net lie outside the axis-cylinder, and concludes that they are, in fact, merely the nuclei of enveloping connective tissue cells, and denies that they can be in any way regarded as nuclei of ganglion cells (9). In support of his statement came the experimental work of Eugling (3), who found that cutting of the nerves going to blood-vessels in the rabbit's ear was followed by a degeneration of the nerve net on these blood-vessels. Any persistence of small traces of network was ascribed to incomplete section of the

DESCRIPTION OF PLATE.

(All preparations made by the intra-vitam methylene blue process.)

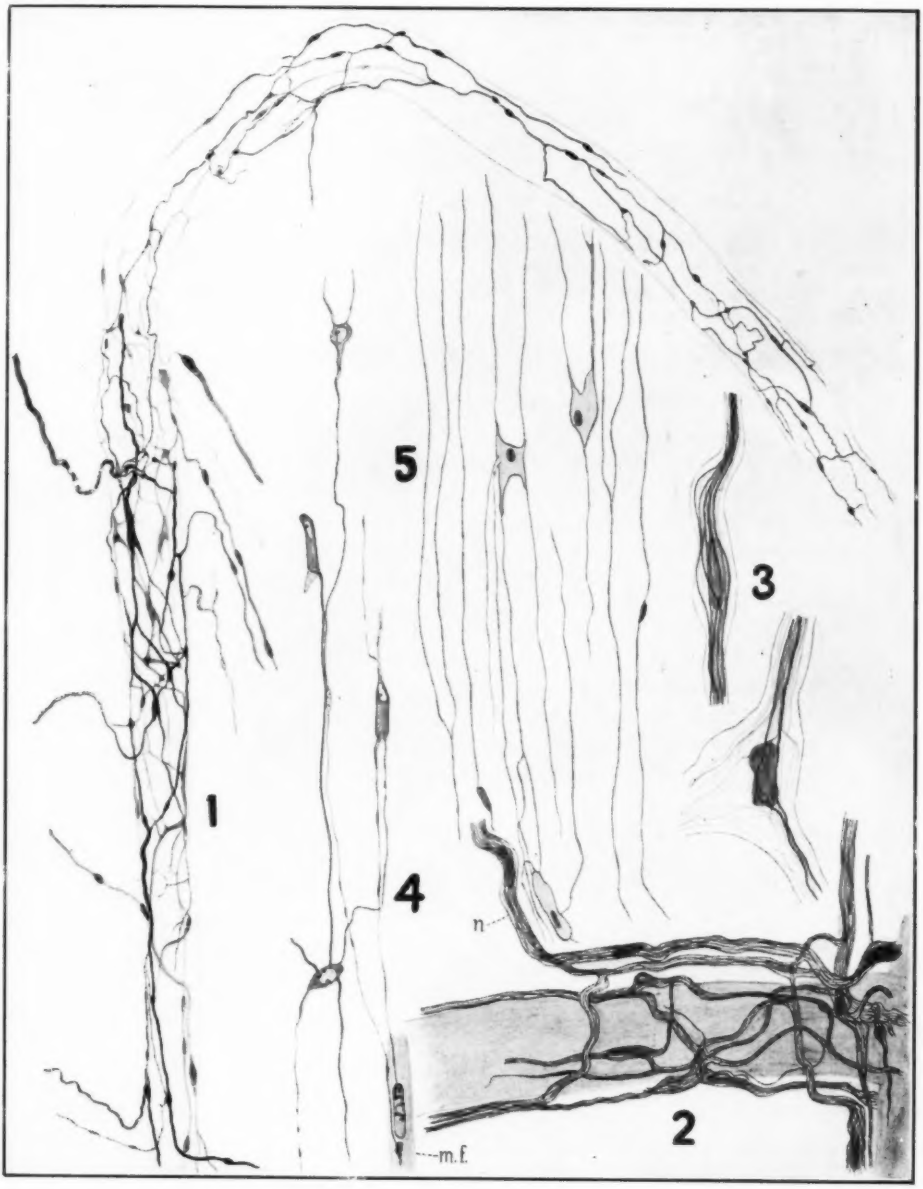
Fig. 1. Nerve net on surface of a small artery, from stomach of frog *(Hyla aurea)*. Length of blood-vessel about 5 mm. Numerous non-medullated fibres are seen terminating upon the nerve net, which forms a *continuous* system over the whole blood-vessel. Nerve net on upper and lower sides indicated by difference in shading.

Fig. 2. Nerve net from small artery on stomach of frog *(Hyla aurea)*, to show continuity of its neurofibrils with those of the main nerve fibre (n). (X 420.)

Fig. 3. Portion of same, showing nuclei surrounded by neurofibrils. Neurofibrils on opposite sides indicated by dark and light shading. (X 850.)

Fig. 4. A portion of nerve net of stomach musculature of frog *(Hyla aurea)*, showing fine nerve fibres terminating by two end-plates, on a plain muscle fibre (m.f.). (X 420.)

Fig. 5. Portion of nerve net in longitudinal plain muscle coat of earthworm. Five nuclei are shown, and a considerable amount of anastomosis between fibres of adjacent cells occurs. (X 420.)


5
3
1
4
n
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m.f.

nerves, an assumption which is probably justifiable. Eugling further observed that in methylene blue preparations of such nerve nets, the "neurofibrils" presented minute varicosities, exactly as do the very delicate non-medullated nerves that are so well seen, for example, in good preparations of Auerbach's plexus. From this he drew the following conclusion: The nerve nets of Bethe are not ganglionated plexuses, capable of local reflexes, but on the contrary they consist of bundles of very fine nerve fibres ("neurofibrils" of Bethe); these are bound together in loose bundles by means of connective tissue cells, whose nuclei Bethe had regarded, erroneously, as ganglion nuclei. This opinion is now widely accepted; for example, Bayliss, in his "Principles of General Physiology," remarks: "Peripheral nerve nets of the kind described above clearly serve only for conduction, and not for origination of nervous impulses."

But, however satisfying the evidence brought forward by Hofmann and by Eugling may at first appear, these conclusions are open to serious criticism. Let us consider first the question of the degeneration of the nerve net, following upon section of the main nerve trunks. It seems to me that it is only with the greatest caution that we can draw any conclusion from the results of the method of Wallerian degeneration, since so many complications frequently arise to obscure the evidence. It is well known, for instance, that frequently peripheral nerve degeneration is accompanied by a degeneration which extends inwards into the spinal cord, resulting in the well-known Nissl degeneration. It is well known also that following on section of a motor nerve to a skeletal muscle, the muscle may undergo very pronounced degeneration; for example, Langley and Hashimoto find (11) that three weeks after section of a motor nerve to a muscle, the muscle may have lost thirty to fifty per cent. of its weight. Similarly, according to Lapinsky (12), degeneration of the vaso-motor nerves of the dog's paw is accompanied by marked degeneration, also, of the small blood-vessels of the paw: there is a marked thickening of the adventitia, a pronounced endothelial proliferation, and a partial degeneration of the muscle fibres. It is clear from these cases that degeneration may extend considerably beyond the nerve affected. When we remember the very intimate relation that must exist between the vaso-motor nerve fibres and the nerve net on the blood-vessels, then clearly the appearance of degeneration in the nerve net can throw but little light upon its nature.

The conclusion of Eugling, that the neurofibrils of the nerve net are really very delicate nerve fibres, is quite erroneous. That they show minute varicosities on staining with methylene blue, similar to those that appear in very delicate nerve fibres, is true. The explanation of this is that the latter consist of only a single neurofibril, and it is this neurofibril which presents the varicosities. That the neurofibrils of the nerve nets are independent nerve fibres, bound together by connective tissue, as Hofmann and Eugling maintain, can be disproved by the following very simple observation: If a good preparation of a

small blood-vessel, with its nerve net stained sharply by the intra-vitam methylene blue process, be made, then the vaso-motor nerves can be observed without any difficulty, becoming continuous with the nerve net (figure 2). Now, as the vaso-motor nerves approach the nerve nets with which they are connected, the neurofibrils become visible with astonishing clearness, and under these conditions they can be seen to be directly continuous with the neurofibrils of the nerve net. Nothing more convincing than the specimens shown in figure 2 could be desired. It seems to me that we must conclude quite definitely from this that the fibrils that traverse the nerve net are not delicate nerve-fibres, as Eugling believed, but that they are directly continuous with the neurofibrils of the nerve-fibres that terminate upon the nerve net.

The question of the nature of the nuclei of the nerve net is more difficult to determine. They are frequently granular in appearance, but do not show the characteristic nucleoli of ganglion cells. The surrounding cytoplasm is also quite devoid of Nissl granules. Nevertheless, there is one observation that I have been able to make, which seems to show that they are to be regarded probably as nerve nuclei. If preparations in which the neurofibrils are clearly stained be examined, it may be seen that the neurofibrils surround the nucleus, and do not lie to one side of it, contrary to what Hofmann states to be the case in Cephalopods. It must be stated at once, however, that this cannot always be seen. Two such cases are shown in figure 3. Now, quite irrespective of our views on the nature and function of neurofibrils, i.e., whether they are the conducting channels of nerves, as Bethe, Apathy, and others believe, or whether they are only supporting elements, or lastly, whether they are mere artefacts, as Mott and Marinesco state, irrespective of all these opinions we can say that they serve to identify nervous tissue. We must conclude, therefore, that the nuclei in the nerve nets are very frequently to be seen surrounded by nervous tissue, and are probably not, therefore, nuclei of enveloping connective tissue cells.[1] However, even if nuclei were entirely absent in nerve nets, we could not state, *a priori*, that the development of reflexes, and even a capacity to initiate rhythmical movements, would necessarily be beyond the powers of such a structure. It is well to bear in mind two important observations bearing on this point. In the well-known experiment of Bethe on *Carcinus maenas*, reflexes were performed by a system of nerve-fibres which had been entirely deprived of nuclei (1). Similarly, Loeb has found that if the *nerve* of a muscle-nerve preparation is bathed in a solution which is deficient in calcium ions, then the muscle supplied

[1] Even this evidence cannot be considered entirely valid, since the nuclear portion of an enveloping connective tissue cell may have sunken into the main mass of the nerve-fibre. It is, in fact, impossible at present to form a definite conclusion on this important point; the nuclei certainly often have the appearance of simple neurolemmal nuclei; but at other times, such as in the longitudinal muscle layer of the frog's stomach (17), it seems quite impossible to consider them as anything other than nerve cells.

by the nerve may pass into a state of strong tetanus (14). In this case the tetanizing impulses are certainly arising in a portion of nerve tissue, which is quite devoid of ganglion cells.

It seems to me that the best evidence in support of the view that a nerve net can, and often does, initiate rhythmical movements, has been provided by those who have sought to deny it. In the experiments of Eugling already quoted, it was found that, once the nerve net on a blood-vessel had undergone degeneration, as a result of section of the vaso-motor nerves, the plain muscle coat of the blood-vessels responded to electrical stimulation only by contraction of that part of the musculature which lay immediately below the electrodes. There was no spreading of impulses; the contraction was purely local. From this it follows that *a strip of plain muscle, deprived of its nerve net, is unable to transmit an excitation wave throughout its substance. Much less then can it originate one.* It seems to me that observations of this kind prove quite clearly that the stimuli for the rhythmical movements undergone by isolated ganglion-free strips of plain muscle must arise in the nerve net, and not in the muscle-tissue itself.

The recent work of Krogh and his co-workers (10) on capillary reactions provides additional evidence in favour of this view. Krogh observed that section of vaso-motor nerves had no effect on the local reactions of small blood-vessels until sufficient time had intervened for these nerves to undergo degeneration. After this responses of blood-vessels to stimuli were strictly localized, and no spreading whatever of impulses occurred. In order to explain these observations the authors had to postulate axon reflexes in a nerve net of the kind described by Bethe.

From these various observations, histological and physiological, we must conclude that the stimuli for rhythmic movements in plain muscle arise, not in the muscle-tissue, as is generally believed, but in its nerve net—the movement is neurogenic, not myogenic.

It is necessary to refer in this place to some important observations by M. R. Lewis (13), which might be considered to disprove the above conclusions. It was observed that isolated embryonic plain muscle cells, when grown in culture, could undergo rhythmic beating, and that if a number of such cells were in contact with one another they could beat in unison, although quite devoid of nerves. It is especially to be noticed about these observations, however, that the cells were not plain muscle cells, but only undifferentiated embryonic cells, similar in appearance to embryonic heart-muscle cells. The problem of co-ordination of movement of these cells in culture is probably identical with the question of the conduction of impulses in the embryonic heart. It might be suggested that here the action currents of the dominant cells (pacemakers) of the tissue excite the neighbouring cells into activity, the stimulus passing rapidly from cell to cell. The well-known "rheoscopic frog" experiment would serve as an

analogy. The slowness of the conduction process in plain muscle excludes this factor as a possible basis for a myogenic origin of rhythmical beating in this tissue.[2]

Note on the Structure and Occurrence of Nerve Nets.

The actual relation of the nerve nets to the plain muscle tissue which they traverse is difficult to observe, and it has at times been stated that they have no direct relation to the muscle fibres, but merely envelop them (cf., for example, the work of Hofmann (9) on Cephalopod material). The difficulty arises from the fact that methylene blue rarely stains the nerve nets and the nerve endings at the same time. In most successful preparations of nerve nets, nerve endings are not seen; while at other times considerable stretches of tissue are observed, in which nerve endings, and the terminal portion of the delicate nerves attached to them, can be seen on nearly every muscle fibre that has stained, the nerve net in this situation remaining unstained. I have, however, occasionally observed direct connection between the nerve net and terminal motor endings. Such a case is shown in figure 4, where the two fibres that end upon the muscle fibre arise from separate parts of the nerve net. In a previous paper I have figured a case in which the two nerves arose from one single nerve cell (17).

In the medusae nerve nets constitute the greater part of the nervous system, lying in intimate association with the musculature of the sub-umbrella, and connected, as Hesse has found (8), with the peripheral sense organs by long conducting fibres. In actinians a similar nerve net occurs (15), although long-distance conducting fibres are also present, arising from peripheral receptors in the epidermis.

In echinoderms, in addition to the centralized nerve ring, there occurs a peripheral nerve net (1), lying in close association with the musculature. In mollusca a subepithelial nerve net occurs. This appears to communicate with the central nervous system, and it is believed that a peripheral nerve net, in intimate association with the musculature, and connected directly with the subepithelial plexus, and also with the central nervous system, exists (1).

In the lower worms (planarians) a nerve net is present; but in the annulata it is usually believed to be absent, the whole of the co-ordinating function being

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[2] Through the courtesy of Dr. Kellaway and Miss Williams, of the Walter and Eliza Hall Research Institute, I have been able to observe a culture of embryonic chick heart tissue, beating actively. The appearance of such a co-ordinated beat is not at variance with what one would expect from these remarks. The whole tissue, or if contraction is confined to part of it, then all the cells of this part contract, so far as the eye can follow, *simultaneously*. The beat presents a marked contrast to the contraction *wave*, so well seen in living plain muscle, and the action current of localized dominant cells might amply account for the co-ordination. At the same time, of course, it is not possible to distinguish by mere inspection such a mode of co-ordination from one having as its basis transmission of an impulse with the high velocity such as we find in striated muscle tissue.

regarded as lodged in the central nerve cord. While it is true that segments of an earthworm, whose ganglia have been removed, are paralyzed, so far as their capacity to partake in the general locomotion of the animal is concerned, the view that such a deganglionated musculature is incapable of very simple automatic movement is incorrect. I find that if the ventral nerve cord is removed from a series of segments, slow and weak contraction waves may still be seen travelling occasionally over such a piece of ganglion-free tissue. It might be concluded that we have before us here a co-ordinated contraction of plain muscle tissue, in the absence of a special conducting system. But here, too, a nerve net, although a much modified one, can be detected. If preparations of the earthworm musculature be made, by the intra-vitam methylene blue method, delicate nerve fibres, running parallel to and between the plain muscle fibres, may be clearly observed. They branch occasionally, and nuclei may be seen in small numbers upon them. If good preparations are obtained, branches of certain of these cells may be seen anastomosing, and being directly continuous, with similar processes from other cells (figure 5). It should be particularly noted, however, that this branching is far less prominent a feature of the peripheral nerves of the earthworm, than it is, for example, of a similar structure occurring in medusae, or in the plain muscle of vertebrates. This is probably correlated with the fact that the earthworm musculature is much more under the influence of, and directly dependent on the control of, the central nervous system, than it is in lower animals; and that as the experiments of Friedländer have shown, locomotion in this animal is not a co-ordinated action of its whole musculature, but rather the co-ordinated action of the musculature of the individual segments (4).

The musculature of insects, which is of the ordinary striated type, is quite devoid of such nerve nets.

In the vertebrates nerve nets are not found in connection with skeletal muscle, but can, I think, be demonstrated in all those situations where plain muscle occurs. Numerous investigators (Dogiel, Golgi, Bethe, and others) have recorded their presence on small blood-vessels. On no structure can they be more clearly seen than on the musculature of large blood-vessels. Yet the occurrence of rhythmical movements in isolated strips of plain muscle from this source, which does not contain anything corresponding to a myenteric plexus, has been taken recently to demonstrate the myogenic origin of these movements (5, 18). In the ureter it is well developed. It is believed not to occur in the retractor-penis muscle of mammals, while, on the contrary, it can be demonstrated here with the greatest clearness (figure 6). In the musculature of the bladder a nerve net is also to be found.

In connection with the nerve net of the intestine, a curious situation has arisen. It is customary to quote the Auerbach plexus as an example of the occurrence of a nerve net in a vertebrate; see, for example, G. H. Parker (15). Nevertheless, this plexus partakes more of the character of a true synaptic nervous

system, although, as Dogiel (2) has found, there occur in the ganglia a certain number of nerve cells, whose dendrites anastomose with one another, thus providing a possible basis for a diffuse conduction in all directions. It is clear, however, that such an action as the myenteric reflex is quite beyond the capacity of an ordinary nerve net.

Fig. 6. Nerve net of retractor penis muscle of dog, from a methylene blue preparation. The specimen is from about 4 mm. length of tissue.

In addition to the Auerbach and Meissner plexuses, there occurs actually within the muscle coat a most pronounced nerve net. It was apparently first observed by Gerlach (6), who believed it to be a plexus of nerve fibres, devoid of ganglion cells, the nuclei of the plexus being nuclei of enveloping connective tissue cells. Schultz (16) described a conducting system within the plain muscle coat, but it is clear from his figure that he was observing the branched connective

tissue cells so prominent in this region. Bethe, however, stated that a true nerve net occurs in the intestine in addition to the well-known plexuses of Auerbach and Meissner (1, p. 122). I have seen the same thing very clearly in methylene blue preparations of the intestine (17), and of the existence of such a nerve net, actually within the muscular coat, and having direct communication with the myenteric plexus, there can be no doubt whatever. This nerve net is, however, always overlooked in present-day text-books on histology, and the nerve fibres originating in Auerbach's plexus are invariably figured as terminating directly upon the fibres of the muscle coat.

From the above review it seems possible to draw one general conclusion: In all those animals in which the musculature is of the plain muscle type, a nerve net occurs in close association with this tissue. In animals whose musculature is of the striated type, such a nerve net is absent. In animals in which some of the muscles are of the striated type and some of the plain type, a nerve net occurs always in connection with the latter, never in connection with the former. And these observations are in agreement with the view that in striated muscle a specialized conducting path is developed within the fibres—the two membranes of Krause—while in plain muscle no such structure is present, the nerve net serving here to conduct the stimulus through the tissue.

SUMMARY.

1. A nerve net is always developed in intimate association with plain muscle tissue, never in connection with striated muscle tissue. Nerve fibres arise from the nerve net, and terminate directly on the muscle fibres.

2. Such a nerve net is capable of serving as a basis for simple reflexes, without the intervention of a central nervous system.

3. Automatic rhythmic movements of isolated, deganglionated plain muscle strips always originate in the nerve net, and never directly in the muscle coat.

4. Co-ordinated action of plain muscle tissue is always neurogenic, never myogenic.

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